DETOXIFICATION OF AFLATOXIN – CONTAMINATED AGRICULTURAL COMMODITIES

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ABSTRACT

Aflatoxin-contaminated commodities can be detoxified by a variety of methods based to some extent on economics and the physical and chemical characteristics of the substance being treated. Electronic and hand sorting of peanuts with ammoniation of contaminated oilseed meals and corn currently appears to offer the most promise. Microbiological decontamination appears to be the least feasible method of choice.

INTRODUCTION

Growing documentation on the presence of mycotoxins, aflatoxin in particular, in foods and feeds indicates the need for practical and economic detoxification processes at least with respect to feedstuffs. In addition to technical and economic considerations, such processes should also reduce toxin concentrations to safe levels, cause no new toxin formation nor result in loss of nutritive value of the treated commodity. Investigations into mycotoxin-detoxification have centered primarily about aflatoxin; there have been a few reports in the literature, with no positive results, on the trichothecenes (Mains *et al.*, 1928; Tuite *et al.*, 1974).

With respect to aflatoxin-contaminated commodities, detoxification processes must take into consideration the usual non-homogeneity of the contamination; i.e., aflatoxin is often localized in only a small proportion of the contaminated product so called "hot spots." This may prove an asset in certain inactivation methods but pose a difficulty in others. Procedures that have been investigated can be arbitrarily divided into three categories: (1) physical; (2) chemical; (3) biological; the first two categories appear to offer the most promise.

PHYSICAL

Separation

Physical separation procedures have proven most successful with peanuts and other nutmeats, since aflatoxin-contaminated

kernels are usually damaged, shriveled, or discolored (Natarajan *et el.*, 1975a). Hence, a combination of sieving and electronic sorting should serve to eliminate most of the undesired kernels and leave the remaining nuts virtually free of aflatoxin. A final hand-picking is used to detect and eliminate those nuts escaping the first two processing steps. The need for both electronic sorting and hand picking is emphasized in a recent publication that indicates either method alone could be inadequate (Dickens and Whitaker, 1975).

Rice appears to offer no problem in the United States, but Schroeder *et al.* (1968) have shown that milling procedures could serve to reduce aflatoxin contamination. However, physical separation methods are generally ineffective for lowering the aflatoxin content of naturally contaminated corn (Brekke *et al.*, 1975a, b).

Physical separation might prove feasible with cottonseed, since there appears to be a good correlation between the presence of aflatoxin and the occurrence of a bright greenish-yellow fluorescence (BGY) in the contaminated seed under ultraviolet light (Ashworth and McMeans, 1966). Electronic sorting devices can be effectively used to eliminate contaminated seed, but economics may not favor this approach. Al alternative would be to use ultraviolet light to detect potentially contaminated lots and segregate these out.

A similar BGY fluorescence may also occur in aflatoxin-contaminated corn (Fennell *et al.*, 1973), and its presence has been used as an "early-warning system" to indicate the need for more comprehensive analyses. However, care and judgment should be exercised in the interpretation of BGY fluorescence because the fluorescing substances are not aflatoxin, and other fluorescing compounds may be present that could lead to mistaken conclusions. In addition, some kernels that appear to be sound and do not fluoresce when whole reveal fluorescence upon cracking. These latter kernels would ordinarily escape detection thus obviating any electronic sorting process. It is best to regard the ultraviolet light procedure as a preliminary screening device to be followed up by recommended analytical aflatoxin analyses should the presence of these toxins be suspected.

Heat

Steaming under pressure has been shown to effect some reduction in aflatoxin content of peanut meal (Coomes *et al.*, 1966), but this approach by itself is probably inadequate. However, processing of milk following pasteuriztion by freeze- or spray-drying does effect reduction of aflatoxin M (Purchase *et al.*, 1972). More promising would appear to be the application of dry and oil roasting to nutmeats, but again, reduction, while significant, may be inadequate to meet FDA recommendations (Lee *et al.*, 1968, 1969; Escher *et al.*, 1973). Some preliminary and promising experiments on dry roasting of corn have been carried out at our laboratory by Engineering research personnel, but considerably more research is required before the efficacy of this approach can be evaluated.

Radiation

Use of gamma rays and ultraviolet would appear to be ineffective (Feuell, 1966; Miyaki *et al.*, 1967). Soft x-rays and electron irradiation at doses that could possibly be effective would result in destruction of the irradiated commodity (Frank and Grunewald, 1970). However, an avenue that has not been explored would be the simultaneous application, for brief periods, of high radiation doses combined with heat that would be available in an atomic pile.

Solvent extraction

This approach has been extensively studied at the Southern Regional Research Center in New Orleans. A variety of polar solvents have been investigated for the reduction or elimination of aflatoxin primarily from cottonseed and peanut meals (Gardner *et al.*, 1968; Rayner and Dollear, 1968; Rayner *et al.*, 1970). Solvents or mixtures of solvents, such as methanol, ethanol, acetone, chloroform, benzene, and aqueous isopropanol, appear to offer promise except that they do affect the nutritive value of the extracted commodity and are somewhat higher in cost than more recently developed chemical detoxification procedures.

CHEMICAL INACTIVATION

Chemical inactivation currently appears to offer the most promising and feasible approach. Examination of the molecular structure of the aflatoxins would indicate that two points of the molecule appear to be most vulnerable to attack, the internal ester of the coumarin moiety and the double bond of the terminal furan when it is present.

Ozonization

Treatment of contaminated peanut or cottonseed meal at 22% moisture and 100 C for 2 hr with ozone effectively destroyed aflatoxins B_1 and G_1 but was comparatively ineffective against B_2, suggesting that the point of attack was the double bond on the terminal furan (Dwarakanath *et al.*, 1968). However, ozonization decreased the nutritive value of treated meal as determined by duckling and rat feeding trials (Dollear *et al.*, 1968).

Hydrogen Peroxide

A promising laboratory method is the treatment of peanut meal at 80 C for 0.5 hr with hydrogen peroxide at pH 9.5 (Sreenivasamurthy *et al.*, 1967). The treatment did not affect the protein efficiency ratio (PER) in rat trials, and no toxicity was exhibited to the chick embryo.

Methylamine

This compound appears to be efficacious in reducing the aflatoxin content of contaminated peanut and cottonseed meals (Dollear *et al.*, 1968; Mann *et al.*, 1971). Methylamine was applied to give a final 2% concentration to meal adjusted to 15% moisture and heated for 30 min at 100 C. There was a reduction in the PER of the treated peanut meal but not of the treated cottonseed meal. Ninety-day rat feeding studies of the latter meal resulted in reversible hyperfunctional enlargement of the liver, and elevated plasma urea and liver tyrosine transaminase. Methylamine does not currently appear to be under consideration for detoxification

treatments, although the extant data indicate that it might be worth additional investigation.

Sodium Hypochlorite

Provocative data have recently been obtained from the treatment by NaOCl of aflatoxin-contaminated peanut protein isolate and defatted peanut meal (Natarajan *et al.*, 1975b). Results showed that the NaOCl concentration and pH were the most important factors involved in reducing high toxin levels to nondetectable amounts; e.g., at pH 8, 0.4% NaOCl reduced aflatoxin B_1 from 725 ppb to trace amounts in ground raw peanuts; at pH 9, only 0.3% NaOCl was required. Similar results were obtained with defatted peanut meal. However, biological trials on the treated commodities have not been carried out. Scale-up experiments are reportedly in progress.

Ammonia

Use of aqueous or gaseous ammonia with or without heat and pressure is currently receiving the most attention in the United States for detoxification of aflatoxin-contaminated cottonseed and peanut meals and corn. Laboratory studies by Masri *et al.* (1969) on ammoniation of contaminated peanut meal containing 709 ppb aflatoxin B_1, moistened to 9.6% and 14.6% at 200 F for 60 min at 20 psig anhydrous ammonia pressure, reduced the B_1 by 96.4% and 97.6%, respectively. Dollear *et al.* (1968) obtained essentially the same results in a slightly modified process. However, the PER of the treated meal fed to rats was 78% that of a high-quality control meal. Nevertheless, the method shows promise compared to other treatments. In addition, the FDA has approved on a trial basis, the use of ammoniated cottonseed meal in ruminant feeds. In large-scale pilot-plant runs, Gardner and his coworkers (1971) determined optimum processing conditions to inactivate aflatoxins in one or more ton lots of oilseed meals by ammoniation. Moisture levels were adjusted to 12.5%, temperature to 235 to 250 F, and amhydrous ammonia injected to reach 45 to 50 psig. At the end of the run, monocalcium phosphate was added to absorb residual ammonia in the meal to give an odor-free product. Aflatoxin content was reduced from 121 ppb to less than 5 ppb. However, there is some loss in nutritional quality as determined by chemical assays. The ammoniated cottonseed meal was eventually fed to lactating cows and none of the milk samples contained any detectable M_1 (McKinney *et al.*, 1973). At least one commercial firm in the United States is using this process on a continuous basis for detoxification of cottonseed meal under a temporary FDA clearance. Cost analyses have not been published on the procedure, and it is hazardous to attempt guesses without the necessary facts such as plant costs, volume of material processed, raw material costs, and marketing evaluations. An excellent review has been published on ammoniation in a French journal (Prevot, 1974).

A similar process utilizing ammonia, but without pressure or high heat, for the detoxification of corn is currently under active investigation at the Northern Regional Research Center in Peoria. Sufficient data have been gathered to make the process appear very promising. The rate and extent of detoxification are dependent upon ammonia and moisture levels and temperature (Brekke *et al.*, 1975c). Optimum practical conditions appear to involve tempering the corn to 17% moisture, addition

of 1.5% ammonia (w/w), and a temperature of about 38 C; under these conditions toxin levels can be reduced from over 1000 ppb to below 10 ppb in about 2 weeks or less. Increasing the temperature drastically reduces the reaction time required. Ammonia-treated corn has been fed to ducklings, broiler chicks, and trout with good results; treated and noncontaminated control corns gave essentially the same biological results.

The process has subsequently been carried out on 2000 bushel lots of contaminated corn which are being used for large-scale biological trials involving poultry and swine for the purpose of FDA clearance of the ammoniation method. The meat from these animals is currently being fed to rats in a multigeneration study as an indirect human safety test. However, it will be several years before the tests and their evaluation are completed, so no recommendations can currently be made.

The fate of aflatoxin in ammoniated commodities has not been determined. However, using a model system in which pure aflatoxin B_1 was reacted with NH_4OH at 100 C, Lee *et al.* (1974) determined that the major product formed lacked the lactone group. The new compound, aflatoxin D_1, molecular weight 286, was nonfluorescent and was formed by decarboxylation of the β-keto acid resulting from lactone ring opening. Aflatoxin D_1 was determined by TLC of the chloroform extract of a highly contaminated peanut meal that was ammoniated under similar conditions.

Microsublimation of partially purified D_1 yielded micro amounts of two additional compounds with m/e of 236 and 256; structures were not assigned (Stanley *et al.*, 1975). More recently, another compound was isolated from a model system using aflatoxin B_1 and NH_4OH under elevated temperature and pressure. The substance had a mol wt 206, mp 145-146 C, and was a nonfluorescent phenol which retained the difuran moiety but lacked the lactone carbonyl and the cyclopentenone ring (Cucullu *et al.*, 1976). A scheme was proposed for the ammoniation of aflatoxin B_1 to produce aflatoxin D_1 and mol wt 206 compound (dihydro-4-hydroxy-6-methoxyfuro-(2,3,6) benzofuran). Biological data on toxicity have not been reported, although Kiermeier and Ruffer (1974) claim that the nonfluorescent compounds formed in alkaline solution are "strongly toxic" to the chick embryo. It appears obvious that there may be contradictory data in the above reports because the German investigators probably isolated the same compounds as the Americans.

Under less drastic conditions, aflatoxin B_1 when stirred with NH_4OH for 21 hr could be recovered unchanged upon acidification (Vesonder *et al.*, 1975). However, with longer reaction times, a nontoxic residue containing a substituted o-coumaric acid resulted. In a more complex system, Beckwith *et al.* (1975) noted that radiolabeled aflatoxin B_1 added to corn grain flour, when treated with ammonia at 25-50 F for periods of 3-30 days, binds covalently and preferentially to corn protein fractions and water-soluble components. In the presence of base, results indicate that two types of associations can occur between the aflatoxin and macromolecular substrate. A reversible type of association results from opening the lactone ring of the B_1 molecule in basic media and can lead to electrostatic and/or

hydrogen bonding interactions with substrate. The irreversible or covalent interaction between B_1 and substrate does not visibly alter the spectral properties of the primary B_1 chromophore (365 nm absorbance). The affixation of this chromophore to much larger molecules in the presence of weak bases implicates the dihydrofurofuran ring system of B_1 as the site of B_1 interaction. A marked reduction or complete loss of toxicity in certain corn fractions containing the primary B_1 chromophore is further evidence indicating the difuran ring system to be the site of interaction. This entire area of interactions between mycotoxins and the menstruum in which they may occur warrants considerably more research.

Formaldehyde and Calcium Hydroxide

Codifer *et al.* (1976) treated peanut meal contaminated with about 600 ppb aflatoxins with HCHO alone and in combination with calcium hydroxide. At 25% moisture in the meal, treatment for 1 hr in a sealed reactor with 0.5% and 1% HCHO plus 2.0% $Ca(OH)_2$ reduced the aflatoxin level to 3 and 1 ppb, respectively; operating temperatures were about 115-117 C. With the reactor in the reflux mode, atmospheric pressure, operating temperature 104-106 C, 20% moisture, 2.5% HCHO alone, or 1.0% HCHO plus 2% $Ca(OH)_2$ reduced aflatoxin to 5 ppb. None of the treated meals were tested biologically, so proper evaluation of the process will require additional research.

BIOLOGICAL INACTIVATION

A few reports have indicated that aflatoxin may be susceptible to microbial attack (Ashworth *et al.*, 1965; Manabe and Matsuura, 1972). Based on these preliminary observations, we screened over 1000 microorganisms for their ability to either destroy or transform aflatoxin B_1 (Ciegler *et al.*, 1966). Only one of the bacteria assayed, a *Flavobacterium*, removed aflatoxin from solution, but the amount of toxin removed was small and we were able to recover the unchanged toxin from the cells by sonication and washing processes (Lillhoj and Ciegler, 1968). Hence, this approach appears to lack commercial feasibility.

Of more potential importance was our finding that aflatoxin B_1 could be converted to aflatoxin B_{2a} by acid-producing molds; B_{2a} is a comparatively nontoxic compound (Ciegler and Peterson, 1968). We later found that the conversion was a nonspecific hydration catalyzed by acids and did not require the presence of the mold. This did suggest that ensiling with its attendant production of acid could possibly inactivate aflatoxin in contaminated corn. However, in practice, there was no reduction of aflatoxin concentration in ensiled corn, possibly for a variety of rather complex reasons (Lindenfelser and Ciegler, 1970). Direct addition of hydrochloric acid to corn was also ineffective in transforming aflatoxin B_1. A kinetic study of the acid catalyzed conversion of aflatoxins B_1 and G_1 to B_{2a} and G_{2a} has been reported (Pons *et al.*, 1972). Manabe and Matsuura (1972) reported about 50% loss of aflatoxin B_1 and G_1 during the early stages of the miso fermentation and attribute it to microbial degradation; however, direct evidence is lacking.

In a very unique experiment, contaminated peanut meal was composted with bark for 40 days (Nilsson and Valdmaa, 1974). After 10 days of composting, both the aflatoxin and most of the mold flora had "disappeared." It is difficult to weigh the practicality of this approach.

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